

pd(N6) in a total volume of 15 μ l. Two μ l of the completed first cDNA strand reaction was then directly used per 12 μ l PCR reaction by adding 10 μ l PCR mix containing 10 pmol each of the mouse/human derived primers KIT1F and KIT7R (5'-TCR TAC ATA GAA AGA GAY GTG ACT C (SEQ. ID No. 3) and 5'-AGC CTT CCT TGA TCA TCT TGT AG (SEQ. ID No. 4), respectively; Moller *et al.* 1996, *supra*), 1.2 μ l 10 x PCR-buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl) and 0.5 U of AmpliTaq polymerase (Perkin-Elmer) incubated with an equal amount Taqstart antibody (Clonetech) at 25°C for 5 min to achieve a hot start PCR. The reaction was covered with 20 μ l mineral oil and thermocycled in a Hybaid Touchdown machine (Hybaid) with 40 cycles at 94°C for 1 min, 55-48°C (touchdown one degree per cycle the first seven cycles and then 48°C in the remaining cycles) for 1 min and 72°C for 1 min. After PCR 2 μ l loading dye was added to each sample which were then loaded on 4% agarose gel (Nusieve/Seakem 3:1, FMC Bioproducts) and electrophoresed with 100V for 80 min. Products were visualised by ethidium bromide staining and UV-illumination.

On page 19, paragraph starting on line 24 and ending on page 20, line 9:

i. PCR to produce DNA Sequencing Template

A 175 bp region including the boundary between exon17 and intron17 of the *KIT* gene was amplified for sequence analysis using forward primer KIT21 (5'-GTA TTC ACA GAG ACT TGG CCG C-3') (SEQ. ID No. 1); and reverse primer KIT35 (5'-AAA CCT GCA AGG AAA ATC CTT CAC GG-3') (SEQ. ID No. 2). PCR was carried out on a DNA thermal cycler (Perkin Elmer 9600) in a total volume of 20 μ l containing 25 ng genomic DNA, 1.0 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 200 μ M dNTPs, 0.5 U AmpliTaq Gold (Perkin Elmer) and 10 pmol of both KIT21 and KIT35 primer. To activate AmpliTaq Gold, initial heat denaturation was carried out at 94°C, 45 sec at 55°C and 45 sec at 72°C. The final extension lasted for 7 min at 72°C. PCR products were cloned into vector pUC18 using the SureClone ligation kit (Pharmacia Biotech).

On page 29, paragraph at lines 3-14:

The PCR primers used were as described below:

KITTM-Nest-F (5'-CTC CTT ACT CAT GGT CGA ATC ACA-3') (SEQ. ID No. 6)

and

KITTM-Nest-R (5'-CGG CTA AAA TGC ATG GTA TGG-3') (SEQ. ID No. 7).

The TaqMan® probes used were: